The Biological Properties of Schick and Dick Toxins.

There is still much of pioneer work to be done in relation to immunity procedures in connection with diphtheria and scarlet fever, and these two papers by Dr. O'Brien and Dr. Joe, submitted at a meeting of the Fever Hospital Medical Service Group on January 27th, 1928, give an idea as to how it is being done in this country.

I.—STABLE SCHICK AND DICK DILUTIONS.

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SCHICK TOXIN.

THE instability of diphtheria toxin, when diluted several hundred times in readiness for the Schick test, has in the past been a disadvantage.

In the earliest work on the subject it is probable that most workers used about 0·3 per cent. of phenol or allied preservative. As shown by Glenny, Pope, Waddington and Wallace (1925), diluted toxin is so unstable in the presence of small quantities of phenol that gentle shaking for twenty minutes, represented by a man walking with a bottle in his pocket, causes detectable deterioration. It was therefore necessary to use this type of dilution immediately after it was made. The next step was to discontinue the use of phenol and use Schick test toxin without a preservative. The danger here was that if any organisms were introduced during manipulation they might grow rapidly. Experience proved that this was a real danger. It was known also that a small change in reaction might seriously affect the diluted toxin; it was therefore advisable to buffer the solution so that the pH could not change. These two objects were achieved by the use of a combination of salts, viz., crystal borax 57, boric acid 84 and sodium chloride 99 parts (Glenny, Pope and Waddington, 1928). A 1·5 per cent. solution of this mixture of salts is made up and autoclaved and used as the diluent for Schick toxin; this solution is isotonic with human blood.

In animal experiments it was found that Schick toxin prepared with this diluent remained of full potency at room temperature for four to six weeks, and, when kept in the cold room, three to six months or more. The amount of boric acid present proved to be sufficient to inhibit the growth of "air contaminants" intentionally introduced in a series of experiments. After we had tested the dilution on a number of volunteers, further comparisons were made, through the kindness of several clinical friends, of the reactions produced by diphtheria toxin diluted in saline and used immediately, and of the buffer salt (B.B.S.) dilution. In the first series 127 tests were carried out; sixty-two patients were positive to both dilutions (amongst these, seven gave a larger response to the B.B.S. than to the saline dilution, and two a smaller); two patients were positive to B.B.S. and negative to the saline dilution.

The next step was to test the stability. Various batches were made up and a number of phials filled, kept either at room temperature (R.T.) or in the cold room (C.R.) and sent periodically for comparison with the ordinary freshly prepared B.B.S. dilution. Dr. E. H. R. Harries, in a long series of observations, found no deterioration in the R.T. phials up to five weeks after their preparation. Some of the C.R. dilutions made up nine months ago are still in use; they gave full-size Schick positive reactions up to six months after being filled; after nine months they still gave good reactions, but these were occasionally smaller than those given by fresh dilutions. One can safely say, as the result of these observations, that the R.T. phials are of full value for two to four weeks, and the C.R. for three to six months. Similar results, not yet published, were obtained by Dr. A. Joe.

In the longest clinical series 151 subjects were tested; of these, 76 were negative to the fresh saline (S) and to the B (=B.B.S.) dilution; 52 gave equal reactions; in 11, B was greater than S; in 5, B was less than S; in 7 subjects discrepancies were found, 6 being positive to B and negative to S, and 1 positive to S and negative to B.

The B.B.S. diluent has been in routine use for about a year, and has given reliable results.

DICK TOXIN.

The next step was to use the same diluent for scarlet fever toxin used in the Dick test. Here the same course was followed. After we had tested the B.B.S. mixture of salts without toxin on volunteers and compared B.B.S. (B) and 0·4 per cent. phenol saline (S) dilutions, a number of clinical friends made further comparisons of the two dilutions. In the first series 125 comparisons were made; of these subjects, 32 were negative to both dilutions and 87 were positive; 5 subjects were positive to B and not to S, and 1 was positive to S and not to B. Of the 87 reactors positive to both dilutions, 61 gave equal reactions with both, and 26 gave greater reactions with B than S. In some of the readings the greater size of the B reaction was striking. In another series of observations, covering 85 subjects, four clinicians reported that B was certainly stronger than S; one found them approximately equal. This led to the hypothesis that the mere act of dilution in saline causes some of the "toxin" to disappear. We, therefore, made up a dilution of 1/1000 toxin with B and dilutions of 1/1000, 1/1500 and 1/250 with S, and endeavoured to find out which strength of S corresponded to 1/1000 B. The dilutions were made up under exactly comparable conditions, the same kind of phial and the same original distilled water being used for all dilutions; they were dispatched through the post in the same parcel.

Dr. Harries made four series of observations, 57 comparisons in all. He found that the 1/1000 B gave in many instances stronger reactions than 1/1000 S, that in many, 1/1000 B was stronger than 1/500 S, and in a small number was approximately equal to 1/250 S. (See also results, in the article following, of Drs. Joe and Swyer.)

It was, therefore, evident that under certain conditions not clearly understood, B dilution 1/1000 was more than twice but not four times stronger than 1/1000 S. What the cause of the disappearance of toxin is we cannot suggest with any confidence. The two obvious possibilities are a change in pH or destruction through shaking in the post. It would require larger numbers of tests to investigate these points. Grave errors may be made if one trusts to small numbers of volunteers in the effort to make accurate comparisons of various solutions, and human volunteers in large numbers are not readily available.

We have used the B diluent as a routine for about a year; Dick toxin so diluted is apparently stable when kept at room temperature for a period of weeks, but not of months.

CONCLUSIONS AND SUMMARY.

Schick Toxin.—As a diluent the mixture of salts described gives a reasonably stable dilution for Schick testing. This can be used with safety for a period of at least two weeks if kept at room temperature.

Dick Toxin.—The dilution of the culture filtrate of streptococcus scarlatinæ with saline may give a dilution half or a quarter as potent as one made with a solution of the salts described.

It is a pleasure to acknowledge constant co-operation and help from Drs. E. H. R. Harries, A. Joe, W. T. Benson, and J. Smith, who did most of the series of observations. We are also very grateful for records of observations from Drs. C. Blake, D. MacIntyre, C. McEntee, E. James, W. Dow, J. Reid, J. Armstrong, W. A. Horne, and Elsie Mann.

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II.—OBSERVATIONS ON DICK TOXIN.

By Alexander Joe, D.S.C., M.D., Ch.B. Edin., D.P.H., D.T.M., and Robert Swyer, M.R.C.S., L.R.C.P., D.P.H., North-Western Hospital, Metropolitan Asylums Board.

I.—Stability of Dick Toxin.

THIS experiment was carried out to determine what deterioration, if any, occurs on the storage of Dick toxin for one year. The toxin tested, X48, B.1910, was one received on December 12th, 1925, and kept at room temperature until the time of experiment, *viz.*, January 24th, 1927. Controls were made with toxins X48, B.2881 and B.2901 which had been received during the previous month. The diluent employed in all the toxins mentioned was the usual normal saline. The results are shown in the following table:—

Number tested	No. of cases which were positive to both aged and fresh toxin	No. of cases which were negative to both aged and fresh toxin
16 (Diphtheria Convalescents aged from 3 to 33)	8 ...	8
15 (Cases of scarlet fever aged from 5 to 29 years, between 3rd and 116th day)	1 ...	14
Total 31	9 ...	22

II.—Comparative Tests Indicating the Effect of Various Diluents on Dick Toxin.

The diluents used were normal saline, hypotonic buffered solution, and isotonic buffered solution.* For this purpose comparative tests with each of these three solutions of toxin were carried out in each case, the strength of the toxin being 1/1000 and the dosage 0·2 c.cm. Positive reactions, their areas, and their intensities were noted. In all, 123 individuals were tested. Of these, 40 gave negative reaction to each toxin. To the isotonic solution 82 were positive, to the hypotonic, 64 were positive, and to the normal saline 53 were positive. In 60 instances the isotonic reaction was largest, in 16 the hypotonic, and in 6 the normal saline. At present we have no explanation to offer for the two latter results, but are convinced that the above figures give a fair representation and confirmation of the relative potencies of the preparations. By a system of marking, in which a comparative numerical value was given to each, it was found that the intensity of the reactions was in the following order; isotonic strongest, then hypotonic, the normal saline being weakest.

* Isotonic buffered solution is the one referred to in the previous paper as B.B.S.; hypotonic solution contained the same salts but contained somewhat less NaCl.

III.—Comparative Tests to Estimate the Increase in Potency of Dick Toxin using an Isotonic Solution as Diluent instead of Normal Saline.

Serial Dick tests were performed on 46 individuals, each of whom was simultaneously tested with a 1/1000 isotonic solution, 1/250, 1/500 normal saline and a heated control. With regard to the latter, in 24 cases an autoclaved isotonic toxin was used, and in 22 a boiled isotonic toxin. No pseudo reaction was observed in any instance. Of the cases tested, 7 were negative to all toxins, and 39 positive to one or more of the reagents. All reactions were measured and the results were as follow:—

Taking the 1/1000 isotonic as standard—
In 29 cases 1/250 saline was larger
,, 7 ,, 1/250 ,, ,, smaller
,, 2 ,, 1/250 ,, ,, equal.

Again taking the 1/1000 isotonic as standard—

In 33 cases 1/500 saline was smaller
,, 3 ,, 1/500 ,, ,, larger
,, 2 ,, 1/500 ,, ,, equal.

In one instance the only toxin to elicit a reaction was the 1/250 saline.

Conclusions.

I.—In the experiment recorded it would appear that Dick toxin diluted 1/1000 with normal saline undergoes no change during a year of storage at room temperature.

II.—The use of buffered hypotonic and isotonic diluents increases the potency of Dick toxin, the greater increase being observed in the case of the isotonic. This increase has been estimated by experiment to be between 200 and 400 per cent.

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